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STRATEGY FOR GENETIC IMPROVEMENT OF ASCOCHYTA BLIGHT RESISTANCE IN CHICKPEA

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FINAL REPORT

Strategy for Genetic Improvement of Ascochyta Blight Resistance in Chickpea (*Cicer arietinum*)"

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Abstract

Ascochyta blight (AB) is a devastating foliar disease of chickpea occurring in Canada and many other growing regions. This research developed strategies to improve field resistance to AB and/or to increase the durability of resistance in chickpea. The first strategy involved genetic and molecular characterization of divergent sources of resistance within the cultivated species that may contain different genes for resistance to AB. The second strategy involved characterization of the related wild annual species to find better sources of resistance. Ten populations including resistant x susceptible and resistant x resistant crosses were used for the genetic studies. Moderate genetic variation for resistance to AB was observed within each population. Progress in genetic improvement for AB resistance was estimated to range from 10% to 36% depending on the population. The segregation pattern revealed that a few major genes segregate in each population. Quantitative trait loci (QTLs) associated with resistance to AB were identified and mapped in four of the resistant x susceptible populations using SSR based linkage map. Two to three QTLs were identified in each population, some of which are common and other unique to a given population. Three QTLs that together accounted for 56% of the total phenotypic variation for AB resistance were identified for ICCV96029/CDC Frontier population. Further, 35 potential sources of resistance to AB were examined for their allelic profile at the chromosome regions that are associated with the resistance to AB. Three major groups were identified among these genotypes. It appears that resistance to AB could be improved by pyramiding the different genes for resistance as revealed by quantitative genetic and molecular analyses. Evaluation of test populations derived from crosses of different resistant parents with distinct alleles at the QTL for resistance to AB demonstrated a genetic gain of around 10–14%. Several populations at different generations were developed by crossing genotypes developed in this project with adapted lines from the CDC breeding program. Eight putative hybrids were rescued from interspecific crosses between cultivated chickpea and accessions of *C. bijugum* and *C. judaicum* that have better resistance to AB. Improvements to the tissue culture protocols are needed in order to be able to use these two wild species as sources of AB resistance genes. In conclusion, this research indicated that further improvement in resistance to AB is feasible in chickpea and combination strategies of exploitation of the variation within the cultivated species and introgression of resistance genes from related wild species could be used to make substantial genetic gain.

Executive Summary

Ascochyta blight is one of the most destructive diseases of chickpea worldwide. This disease is considered as the most important constraint to chickpea production in the Prairies and elsewhere, resulting in a 100% yield loss in case of severe infection. In this research, we investigated the feasibility of improving resistance to AB through a) pyramiding resistance genes from within the cultivated species and b) introgression of resistance genes from related wild species. The first aspect involved characterization the resistance in the cultivated species that may contain different genes for resistance to ascochyta blight. This will allow breeders to select diverse resistance genes from different sources and to accumulate those resistance genes in elite cultivars to enhance the levels and/or durability of resistance to AB. The second aspect involved characterization of the annual wild relative species of chickpea to find better sources of resistance followed by transfer of the resistance into the cultivated species.

In the first strategy, both conventional (quantitative) genetics and molecular approaches were employed to characterize the parents and 10 populations derived from a series of crosses between a susceptible line (ICCV 96029) and each of four moderately resistant varieties (Amit, CDC Frontier, FLIP 97-133C and ICC 12512-1), as well as 6 possible cross combinations among the resistant lines. Moderate genetic variability for resistance to AB was found within each population. A few genes with moderate to large effects segregated in the populations studied. Progress in genetic improvement for AB resistance was estimated at around 36% from the mean population for progeny derived from susceptible x resistant crosses and around 10% from the mean population for progeny derived from resistant x resistant crosses.

SSR linkage maps for the four susceptible x resistant cross populations were developed. The ICCV96029/CDC Frontier map, which consisted of 144 SSRs and one morphological marker, covered 1,285 cM of the chickpea genome. For this population three QTLs that simultaneously accounted for 56% of the total phenotypic variation for AB resistance were identified. Two of the QTL were common with the previously published QTL for AB resistance in other populations; whereas one QTL on linkage group 3 (LG3) was unique to the current population. Two QTLs for resistance to AB were identified in each ICCV96029/FLIP97-133C and ICCV96029/IC12512-1 populations. The same QTL were also identified in the ICCV96029/Amit population by means of analysis of variance on single markers. These were consistent with the previous reports.

Potential new sources of resistance to AB collected from international chickpea breeding centers were examined for their allelic profile at specific chromosomal regions that are associated with resistance to AB. This procedure potentially differentiates germplasm that may possess different resistance genes. The results demonstrated that a few landraces from the former Soviet Union had SSR alleles that were common among the kabuli breeding lines and cultivars. The cluster analysis differentiated three major groups in the chickpea genotypes evaluated. Disease evaluation on three test populations derived from crosses of parents with distinct genetic profile at the QTL for resistance to AB suggested

a genetic gain of around 10–14 % per generation by crossing moderately resistant genotypes.
A total of 85 F_2 populations and 156 F_4 and F_5 families were developed by intercrossing genotypes developed/identified in this project and by crossing them with adapted breeding lines from the CDC breeding program. These materials were integrated into the CDC chickpea breeding program. First field evaluation was done in 2007. Selected lines will be evaluated in the 2008 growing season together with other breeding materials.

In collaboration with Dr. Monika Lulsdorf's lab, we continued to evaluate potential new sources of resistance to AB from the wild species of chickpea. Eight putative hybrids were rescued from crosses involving accessions of *C. bijugum* and *C. judaicum;* two of the wild species that have better resistance to AB. Protocols for developing hybrid plants are being developed.

TECHNICAL REPORT

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop world wide, accounting for about 15% and 18% of the world's total pulse acreage and production, respectively (FAO, 2006). This crop has recently been introduced to western Canada, and holds an important position in the pulse economy in the region. There is a high potential for future expansion in chickpea production in western Canada because of its high demand especially in export markets and a relatively high return to growers.

In western Canada as well as many other production regions, the chickpea crop is often affected by the foliar disease ascochyta blight, caused by *Ascochyta rabiei.* This disease is considered the most important constraint to chickpea production in the Prairies and elsewhere, resulting in 100% yield loss in case of severe infection (Chang *et al.*, 2007). Ascochyta can infect all above ground parts of the chickpea plant and occurs throughout the growing season, revealing the importance of the disease (Jayakumar *et al.*, 2005). As a result, successful chickpea production in many growing areas nowadays depends on effective management of the ascochyta disease.

Application of fungicides for the control of ascochyta blight may not be cost-effective due to the need for repeated applications (Chongo *et al.*, 2003) and insensitivity of the pathogen population to some of the fungicides (Cho *et al.*, 2004; Chang *et al.*, 2007). Development and use of resistant cultivars is considered the most viable options for the management of ascochyta blight in chickpea and a number of cultivars with improved level of resistance to the disease have been developed in Canada and elsewhere (eg. Muehlbauer *et al.*, 2003; Vandenberg *et al.*, 2003; Warkentin *et al.*, 2005). The use of ascochyta resistant varieties will help to increase and stabilize production levels.

Evaluation of world chickpea germplasm collections revealed that only partial resistance to AB exists in the primary gene pool (Singh and Reddy, 1993) and chickpea varieties

developed from these through breeding are only fair to moderately resistant to the disease. Chickpea breeding programs around the globe have been working on improving field resistance to AB and different strategies are being employed. Some of these strategies include identification and pyramiding race/pathotype-specific resistance genes, use of mutation breeding, and transfer of resistance genes from related wild *Cicer* species. A combination of strategies may be required to make substantial improvement in resistance.

Genetic studies on AB resistance in chickpea have produced variable results, partly due to the variation in the parental genotypes used, location and seasonal effects and method of assessment of the disease reaction. Singh and Reddy (1983) reported that resistance was governed by one dominant gene in four parental genotypes and a single recessive gene in the fifth one. Analysis of data from multiple generations (F_2, BCP_1, BCP_2 and F_3) by Tewari and Pandey (1986) revealed that three independent genes conferred resistance to ascochyta, two dominant and one recessive. Dey and Singh (1993) found six genes, five dominant and one recessive, dispersed in the different parental genotypes which controlled resistance to AB. The authors also noted inter-allelic interaction among these genes in determining resistance to the disease. A more recent study from Tekeoglu *et al.* (2000) using three recombinant inbred line populations derived from intraspecific and interspecific crosses indicated that three recessive and complimentary major genes with several modifiers determined ascochyta resistance. It appears from these and several other reports that resistance to ascochyta blight is under complex genetic control, with various genes having a major or minor independent effect and in some cases interacting with each other in determining the phenotype.

The existence of different ascochyta resistance genes distributed across the different sources of resistance implies that field resistance to the disease could potentially be improved by pyramiding the genes, provided that these genes act in a complementary fashion. However, progress in this effort would be hampered by difficulty in distinguishing genotypes carrying different genes for resistance based on phenotype alone, especially when minor genes are involved and when there exist inter-allelic interactions. Quantitative trait locus (QTL) mapping by means of molecular markers and pyramiding the favorable genes with the assistance of these markers could be a feasible strategy to enhance resistance to AB in chickpea. The use of molecular markers would certainly facilitate the transfer of resistance genes between genotypes.

Identification of QTLs for resistance to AB has been the subject of interest to many recent authors. Santra et al. (2000) identified two QTLs in an interspecific population derived from a cross of *C. arietinum* x *C. reticulatum*. Tekeoglu et al. (2002), using the same RIL population, integrated simple sequence repeats (SSR) markers into the map region where the two QTLs were located. Flandez-Galvez *et al.* (2003) showed that the genetic control of ascochyta blight was quite complex as they identified six QTLs for this disease distributed in three regions of the chickpea genome. Taken together, these studies indicated that the QTLs for resistance to ascochyta are localized on LG2, LG4 and LG8. QTLs for ascochyta resistance on one or more of these LGs were confirmed by other research groups later (Cho *et al.*, 2004; Cobos *et al.*, 2006; Iruela *et al.*, 2007;

Tar'an *et al.*, 2007). Additional QTLs for resistance to ascochyta on LG3 (Tar'an *et al.*, 2007) and LG6 (Cho *et al.*, 2004; Tar'an *et al.*, 2007) were also reported. It appears from these studies that several QTLs on the chickpea genome contribute to AB resistance. These may have resulted from the complex nature of the pathogen and also from the different resistant parents used by the various research groups. However, from a chickpea breeding point of view there is a need to scrutinize these findings and pinpoint the key QTLs having significant effects across different gremplasm and environments. According to Stuber *et al.* (1992), the efficiency with which a plant breeder could manipulate QTL depends on the consistency of the QTL across germplasm and on the effects across environments.

In the absence of a high level of resistance to ascochyta blight in the cultivated chickpea, some attempts have been made over the last two decades to find alternative sources of resistance in the related wild species. Accordingly, some better resistances were identified within accessions of *C. bijugum* and *C. judicum,* whereas moderately resistant accessions were found within*C. reticulatum, C. pinnatifidum* and *C. echinospermum* species (Stamigna *et al.*, 2000; Collard *et al.*, 2001; Nguyen *et al.*, 2005; Pande *et al.*, 2006). Some of the *C. bijugum* accessions reported by Collard *et al.* (2001) were reported to be completely resistant to the disease. Given the importance of AB, it is worthwhile to screen and identify additional sources of resistance under the target environment and against the local isolates of the ascochyta pathogen for use in the interspecific resistance breeding programs.

The research themes for this project were as follows:

1. Determination of the inheritance and allelic relationship of AB resistance genes in chickpea

2. Identification and mapping of QTL conferring resistance to AB in different sources of resistance

3. Diversity analysis based on the SSRs at the QTL for resistance to ascochyta blight

4. Identification and transfer of ascochyta resistance genes from related wild species to the cultigen

5. Integration of improved resistance into the CDC chickpea breeding program

THEME I: Inheritance and allelic relationship of ascochyta blight resistance genes in chickpea

Materials and methods

With the aim to determine the genetic basis of resistance to AB, four divergent moderately resistant genotypes CDC Frontier, Amit, ICC 12512-1 and FLIP 97-133C were crossed to a highly susceptible genotype ICCV 96029. CDC Frontier is an improved cultivar developed at the Crop Development Centre, University of Saskatchewan. Amit (formerly known as B-90) is a selection from a landrace from Bulgaria released by Terramax. ICC 12512-1 is an Indian origin landrace obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). FLIP 97-133C is a line developed from (FLIP91-123C/FLIP84-79C)/FLIP 90-127C crosses and obtained from the International Centre for Agricultural Research in the Dry Areas (ICARDA). Further, the resistant parents were intercrossed in all possible combinations to determine the allelic relationships of the resistance genes in the four parents. A single F_1 plant from each of the ten crosses, i.e. four resistant x susceptible and six resistant x resistant, was grown in the greenhouse and advanced to F_2. In order to get enough F_2 seeds for evaluation, the F_1 plants were multiplied clonally by stem cuttings, and at harvest the seeds from different plants of the same genotype were pooled.

The parents, F_1 and F_2 generations were evaluated for reaction to AB in the greenhouse. Three replications were used for each F_2 genotype by using stem cuttings as indicated above and the experiment was laid out in a Completely Randomized Design. Two weeks after germination, the plants were inoculated with an aggressive isolate of *A. rabiei* ar68-2000 selected from a collection of a large number of isolates from commercial chickpea fields in Saskatchewan. Immediately after inoculation the plants were transferred to a mist chamber and kept at relative humidity of about 100% for 48 hr to facilitate infection. Then the plants were transferred to the greenhouse benches and disease was scored on a 0 to 9 scale at three weeks after inoculation (Chongo *et al.*, 2004).

Analysis of variance was carried out on disease score using PROC GLM of SAS (SAS Institute Inc., Cary, NC, USA). The frequency distribution of the AB ratings was illustrated graphically based on the means of the three replications for each genotype. Also, the heritability of resistance to AB and genetic advance through selection were computed.

Results and Discussion

There were highly significant differences among F_2 genotypes within each population in AB ratings, indicating that some genes conferring resistance to the disease were segregating in each population (Table 1). Moreover, the observation of significant differences within the resistant x resistant cross populations showed that separate genes for resistance to AB were carried by the different sources of resistance. This was supported by the evidence from the distribution of the F_2 populations shown in Fig. 1 in

that the four F_2 populations derived from different resistant parents differed from each other in mode, median and range of AB ratings. It therefore, seems that there is a potential to improve field resistance to AB in chickpea through pyramiding resistance genes carried by the different resistant parents.

The distributions of AB ratings in the F_2 populations from susceptible x resistant crosses were continuous (Fig. 1). However, the Shapiro-Wilk test showed that the distribution of the data in each population deviated from normal distribution ($P \leq 0.05$). The majority of the progeny in the ICCV 96029/FLIP 97-133C population fell within the susceptible rating range, whereas the distributions of the other three populations were skewed towards the resistant parents. It appears that there probably were some major genes related to resistance to AB segregating in each population; however the distribution was still continuous due to the involvement of other minor genes and environmental effects.

Moderate genetic coefficient of variations (GCV) existed among the populations derived from susceptible x resistant crosses (mean GCV = 49%) and resistant x resistant crosses (mean GCV = 20%) (Table 2). At 5% selection intensity, the predicted genetic gain (as a percentage of the population mean) was 36% for populations derived from susceptible x resistant crosses and 10% for populations derived from resistant x resistant crosses. For the crosses among the resistant lines, the highest genetic gain (14%) was found in the ICC 12512-1 x CDC Frontier cross, suggesting that there were some different alleles for resistance to AB in these two varieties. In contrast, the lowest genetic variability (12%) and genetic gain (3%) was observed in the population derived from a cross between FLIP 97-133C x CDC Frontier suggesting that most alleles were in common in these two parents. Our molecular data also demonstrated that FLIP 97-133C and CDC Frontier shared the majority of alleles in the QTL regions associated with the resistance to AB.

Table 1. Mean squares of ascochyta blight rating in ten F_2 populations of chickpea

Cross	df		Mean squares	
	F_2 genotypes	Error	F_2 genotypes	Error
Susceptible x Resistant				
ICCV 96029 x CDC Frontier	186	357	4.05**	0.79
ICCV 96029 x Amit	202	383	3.14**	0.90
ICCV 96029 x ICC 12512-1	179	321	2.92**	0.39
ICCV 96029 x FLIP 97-133C	153	272	6.07**	0.73
Resistant x Resistant				
CDC Frontier x Amit	162	270	0.93**	0.58
CDC Frontier x ICC 12512-1	198	233	1.23**	0.41
CDC Frontier x FLIP 97-133C	245	387	1.34**	0.43
Amit x ICC 12512-1	216	377	1.20**	0.49
Amit x FLIP 97-133C	177	175	1.80**	0.66
ICC 12512-1 x FLIP 97-133C	245	387	1.34**	0.43

** - indicates a highly significant differences at $P \leq 0.01$.

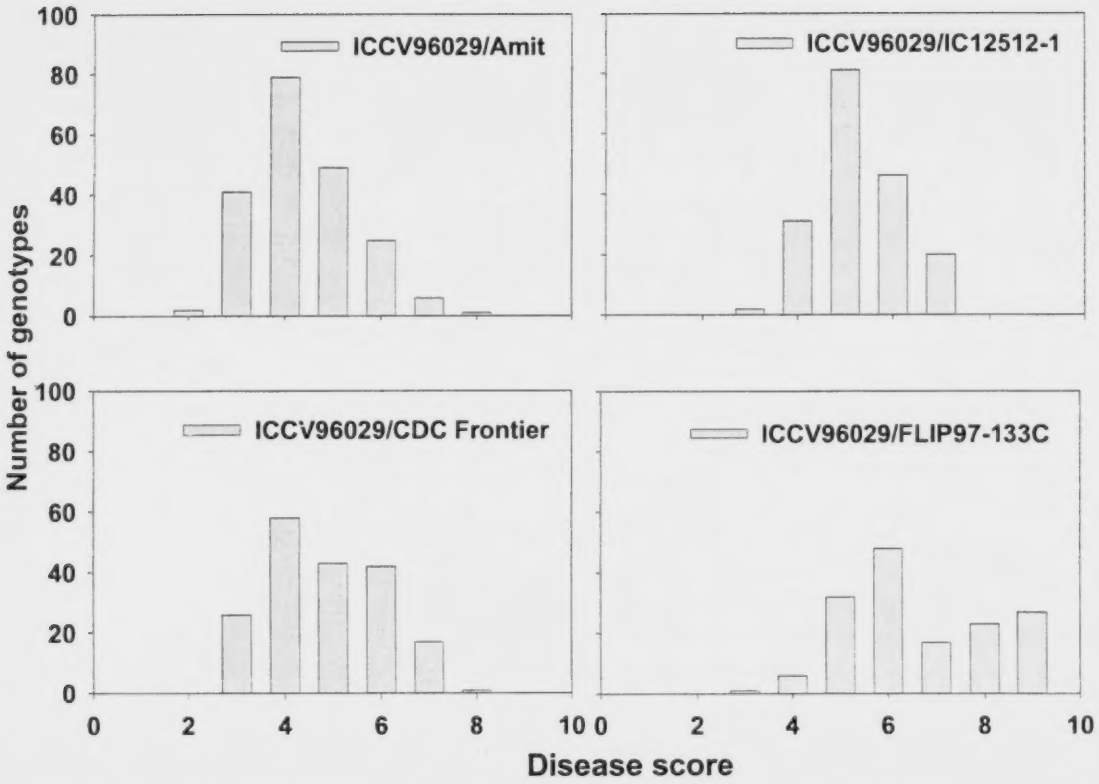


Fig. 1. Frequency distribution of ascochyta blight ratings (1-9 score) in four F_2 populations of chickpea

Table 2. Mean ascochyta blight ratings, genetic coefficient of variation (GCV), heritability (H^2) and predicted genetic advance (GA) of 10 F_2 populations of chickpea

Cross	Mean	GCV (%)	H^2	GA (%)
Susceptible x resistant				
ICCV 96029 x Amit	5.1	41	0.45	27
ICCV 96029 x ICC 12512-1	5.0	42	0.45	28
ICCV 96029 x FLIP 97-133C	6.7	65	0.71	55
ICCV 96029 x CDC Frontier	5.2	47	0.58	34
Resistant x resistant				
Amit x CDC Frontier	4.6	16	0.16	7
ICC12512-1 x Amit	4.8	22	0.32	11
ICC12512-1 x CDC Frontier	4.7	24	0.40	14

ICC12512-1 x FLIP97-133C	6.1	22	0.41	12
FLIP97-133C x Amit	5.9	25	0.37	12
FLIP97-133C x CDC Frontier	5.6	12	0.10	3

THEME II: Identification and mapping of QTL conferring resistance to ascochyta blight in four populations of chickpea

Materials and methods

The four populations ICCV 96029/CDC Frontier, ICCV 96029/Amit, ICCV 96029/ICC 12512-1 and ICCV 96029/FLIP 97-133C indicated above were used for this study. F_1s were advanced to F_2 and then F_2 populations were evaluated as outlined in Theme I above.

For marker analysis, young leaves were harvested from F_2s and parental genotypes and these were freeze-dried for 24 hr. Then DNA was extracted following the CTAB protocol and quantified (Doyle and Doyle, 1987). A large number of SSR primers were screened on the parents and those that showed polymorphism between the two parents were subsequently run on the F_2 populations in each cross. A total of 340 primers were tested on the ICCV 96029/CDC Frontier population, of which 136 showed polymorphism between the two parents. Some of the primers had multiple bands and a total of 144 markers were mapped on this population. About 45-70 markers were mapped in each of the other three populations. The polymerase chain reaction (PCR) for these analyses contained 5 ul of 10ug/ul DNA, 16.3 ul dH_2O, 2.5 ul buffer, 1ul dNTP's and 0.2 ul Taq DNA and the PCR product was separated on a 6% polyacrylamide gel with silver staining. The bands observed were scored using a uniform code for resistant parent, susceptible parent and heterozygotes.

The data were analyzed using MAPMAKER/EXP program version 3.0 (Whitehead Institute for Biomedical Research, Cambridge, MA). Linkage groups of the markers were established using a LOD (logarithm of odds ratio) score of >3.0. The QTL location and effect were estimated by composite interval mapping using the QTL Cartographer program (North Carolina State University, Raleigh, NC).

Results and Discussion

One hundred and forty four SSR markers were assigned to 8 linkage groups with a combined linkage distance of 1,285 cM for the ICCV 96029/CDC Frontier population (Fig. 2). To the best of our knowledge, this genetic map contains the largest number of SSRs from an intraspecific population of chickpea available to date. The average linkage distance between pairs of markers in all linkage groups was 8.9 cM. The maximum distance (28.6 cM) separating two markers occurred on LG4 between the morphological marker (*fc*) and TR11, and the minimum distance between two markers was 0.8 cM on LG7 between $TAA104_{(290)}$ and TA140.

The genetic maps for ICCV 96029/FLIP 97-133C, ICCV 96029/IC 12512-1 and ICCV 96029/Amit were less saturated, but similar to the ICCV 96029/CDC Frontier map in the

position of the markers. Moreover, the SSRs that were common between the current maps and the previous maps (Winter et al. 2000; Tekeoglu et al. 2000; Udupa and Baum 2003) were placed on the same linkage groups, but with slightly different orientation and distance (Figs. 2-5). In contrast to the partial map reported by Cho et al. (2004), the SSRs on LG2 and LG6 were kept in two separate groups, even after lowering the LOD threshold to 2.0 in the grouping command during linkage analysis across all the four populations.

Significant associations between SSR markers and putative QTL for ascochyta blight reaction were found on three linkage groups in ICCV 96029/CDC Frontier population (Table 3; Fig. 2). One QTL each on LG3, LG4 and LG6 accounted for 13%, 29% and 12% of the total estimated phenotypic variation for the reaction to ascochyta blight, respectively. Together, these loci accounted for 56% of the total estimated phenotypic variation. For each genomic region, the allele from CDC Frontier contributed additively to the resistance. The QTL on LG4 flanked by TA2-TA146 co-localized with the QTL reported by Tekeoglu et al. (2002), Udupa and Baum (2003), and Cho et al. (2004). The QTL on LG6 between TA80 and TA22 was near the previously reported region associated with the major gene for resistance in the map of Cho et al. (2004). The QTL on LG3 between TA64 and TS19 of the current map appears to be unique to our population.

Two other QTLs, one on each LG2 and LG4, were identified for the ICCV 96029/FLIP 97-133C population (Fig. 3). The QTL on LG2 was localized between TA59-TS82 and was at the same position as the QTL reported by Iruela et al. (2007) from the ILC3279 × WR315 population. The QTL on LG4 corresponds with one of the QTLs reported by Cho et al. (2004) from PI359075 (blight susceptible) × FLIP 84-92C (blight resistant) population.

In the ICCV 96029/ICC 12512-1 population, two regions on LG1 and LG4 showed significant association with AB resistance (Fig. 4). The QTL on LG1 was tightly linked to TA203 and located between H4G07(228) and TR43, whereas the QTL on LG4 was flanked by TA89 and TS72.

For the fourth population ICCV 96029/Amit, no genomic region had above threshold association with AB resistance, likely due to large gaps between the marker loci analyzed in this population. However, based on a one-way ANOVA, the two QTL regions identified for CDC Frontier on LG3 and LG4, showed significant effects on the resistance in ICCV 96029/Amit population.

In summary, a few QTLs for resistance to AB distributed across different sources of resistance were identified, which implies that field resistance to AB could be improved by pyramiding these QTLs.

Table 3. Location, interval, genetic effects and percentage of the phenotypic variation accounted for by the QTL for ascochyta blight resistance in 186 F_2 plants of chickpea derived from a cross between ICCV96029 and CDC Frontier.

Linkage group	Interval	Interval length (cM)	Nearest locus to the max. LOD peak	Max. LOD score	Genetic effects			% variation (R^2)
					a§	d	d/a	
LG3	TA64-TS19	11.6	TA64	4.27	0.52	0.04	-0.077	13.4
LG4	TA2-TA146	21.5	TS54	9.58	0.97	0.02	0.021	29.4
LG6	TA80-TA22	5.6	TA176	3.88	-0.46	0.02	-0.043	11.6
						Total variation †		55.7

§The value associated with the ICCV96029 allele. A positive value means that the ICCV96029 allele increased the score of the disease reaction.

†The estimated amount of phenotypic variation simultaneously explained by all markers

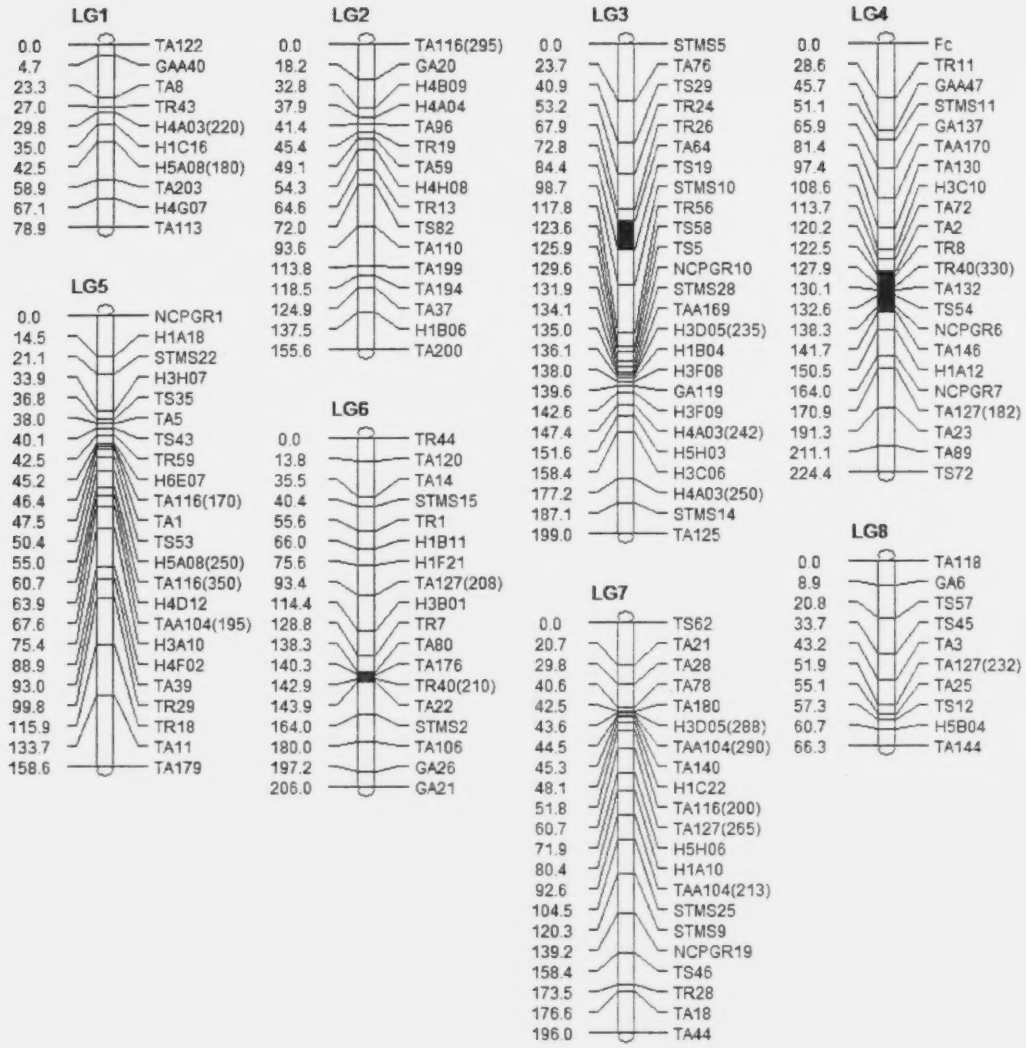


Figure 2. SSR linkage map of chickpea based on 186 F_2 plants derived from a cross between ICCV96029 and CDC Frontier. Linkage distance (cM) is indicated on the left of each linkage group. The locations of the QTL for ascochyta blight resistance are indicated as red boxes.

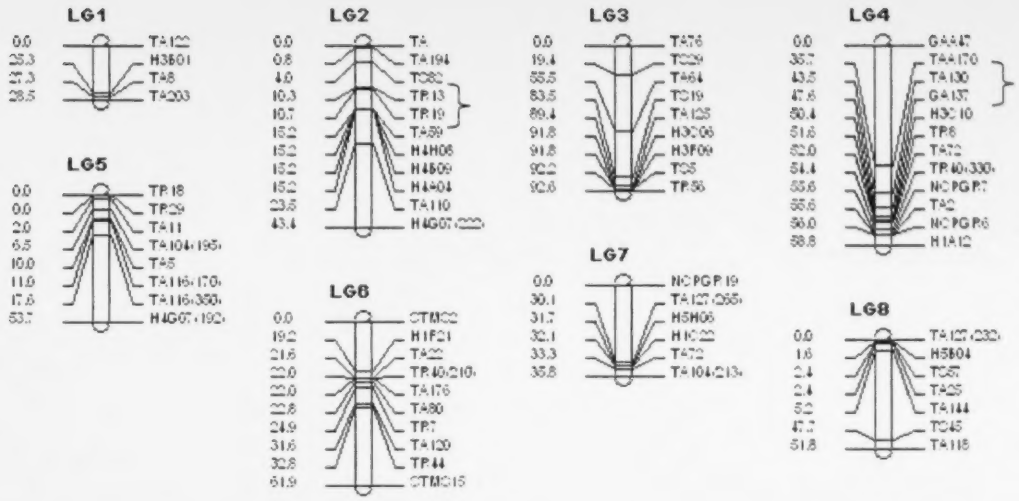


Fig. 3. SSR linkage map of chickpea obtained from ICCV 96029/FLIP 97-133C F_2 population. Numbers on the left side are distances (cM) between markers. Braces show the QTL for ascochyta blight resistance.

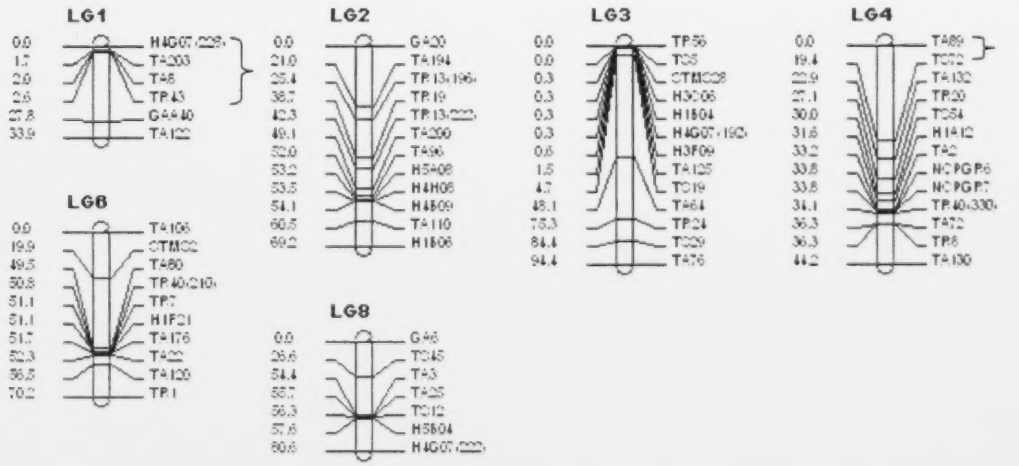
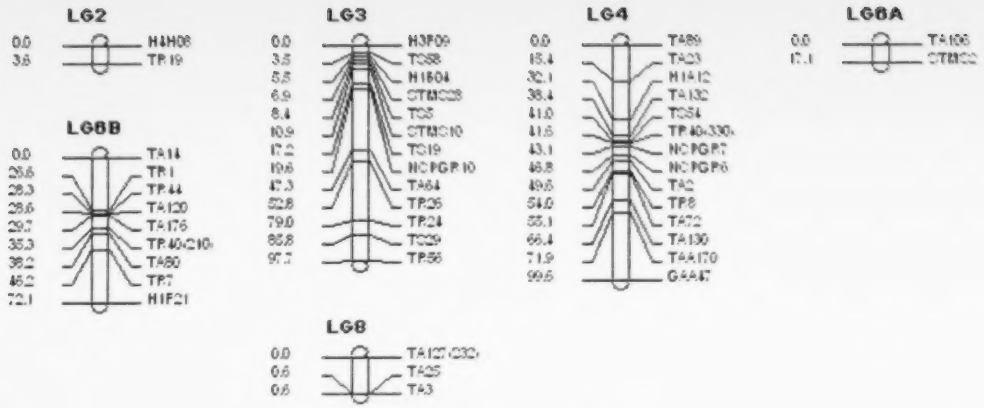
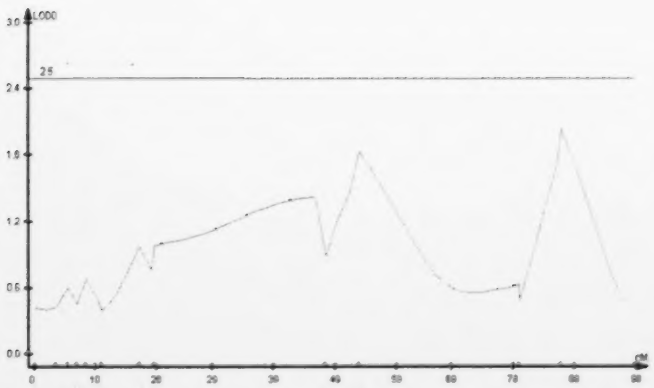


Fig. 4. SSR linkage map of chickpea obtained from ICCV 96029/ICC 12512-1 F_2 population. Numbers on the left side are distances (cM) between markers. Braces show the QTL for ascochyta blight resistance.

A)



B)



C)

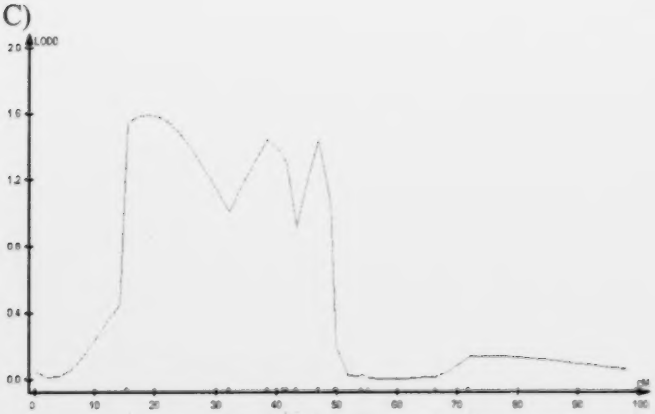


Fig. 5. SSR linkage map of chickpea obtained from ICCV96029/Amit F_2 population (A), and LOD scores of markers on linkage groups 2 (B) and linkage group 4 (C).

THEME III. Diversity analysis based on the SSRs at the QTL for resistance to ascochyta blight

Materials and methods

Potential new sources of resistance to AB from the germplasm collections available from international chickpea breeding centers were screened using a Saskatchewan AB isolate (ar68-2001) and were evaluated by molecular approaches to distinguish whether different accessions potentially carried different genes for resistance. We applied the information from the previous mapping and QTL studies to compare the allele profile of 35 chickpea genotypes differing in reaction to AB, plus two susceptible checks. We used 17 SSR markers surrounding the loci associated with the resistance to AB from the previous QTL studies (Flandez-Galvez *et al.* 2003; Cho *et al.* 2004; and Udupa and Baum 2003) to identify lines with putatively novel resistance alleles. These SSRs were distributed over five regions (LG1, LG2A, LG2B, LG4 and LG8) on the maps reported by Flandez-Galvez *et al.* (2003), Cho *et al* (2004) and Winter *et al.* (2000). In our SSR map developed using ICCV 96029 x CDC Frontier population, the LG2 and LG4 were equal to LG2A and LG4 of Cho *et al.* (2004), respectively. The LG1 of Flandez-Galvez *et al.* (2003) map and the LG2B of Cho *et al.* (2004) map correspond to LG8 and LG6, respectively, of our map.

Results and Discussion

The 17 SSRs used in the analysis detected 2 to 13 alleles (mean = 7.6) across the 37 genotypes and polymorphic information content (PIC values) ranged from 0.47 to 0.87 with mean value of 0.71. On average the SSR loci on LG4 contained fewer alleles compared to the loci on LG2B (Table 4). The average PIC value of the SSR markers on LG4 (0.64) was much smaller than the average PIC value (0.82) of the markers in LG2A and LG2B combined. The associations among the 37 genotypes based on the first two principal coordinates are presented in Fig. 6. The first and second principal coordinates accounted for 19.8 % and 9.7% of the total variation, respectively. This analysis grouped the chickpea genotypes with similar SSR alleles. This analysis confirmed that CDC Frontier and FLIP 97-133C shared the majority of alleles for resistance to AB (Fig. 6). Several genotypes with distinct SSR alleles were identified in the biplot of the first and the second coordinates.

Three segregating populations were developed by crossing parental lines with distinct SSR alleles at the QTL for ascochyta blight resistance. Figure 7 shows the distribution of the disease scores in these populations. Transgressive lines both with enhanced levels of resistance and higher disease scores than the parents were found in each of the F_2 populations derived from crosses between the moderately resistant parents. For population A, the disease score of the F_2 plants ranged from 3.6 to 6.8 (mean = 4.4), while the disease scores of ICC 12004 and FLIP 84-92C were 4.4 and 4.9, respectively. In population B, the scores of ICC 4475 and CDC Frontier were 4.4 and 4.7, respectively. Disease scores ranging from 3.5 to 7.0 were observed among the F_2 plants derived from ICC 4475 and CDC Frontier cross. The same trend was also observed on population C derived from ICC 3996 x Amit cross from which disease scores ranged from 3.4 to 7.0. These results indicated that there were different minor genes for

resistance with additive gene actions in each of the two parental lines in populations A, B and C. Further quantitative analyses suggested a genetic gain around 10–14% per generation with 5% selection intensity on the populations derived by crossing moderately resistant genotypes. These results suggested that it is possible to develop chickpea cultivars with a higher level of resistance by accumulating resistance alleles from different sources that are genetically distant.

Table 4. Summary of 17 SSR loci associated with QTLs for resistance to AB in different linkage groups of the chickpea genetic map

Linkage group (Interval length)	SSR Locus	Number of alleles	PIC	Amplicon size range (bp)
LG1[a]	STMS28	6	0.54	230-252
	TS12	13	0.64	245-300
LG2A[b]	GA20	7	0.83	130-205
	GA16	8	0.84	230-275
LG2B[b]	TA37	5	0.69	258-300
	TR19	11	0.87	206-274
	TA22s	11	0.86	192-280
	TA176s	12	0.82	210-280
LG4[c]	GAA47	2	0.47	154-170
	TA130	7	0.60	180-230
	TR20	7	0.62	148-178
	TA72	8	0.68	220-256
	TA2	6	0.79	130-182
	TS72	7	0.69	230-295
LG8[c]	TS45	7	0.68	224-250
	TA3	4	0.69	260-294
Unassigned	TA46	6	0.51	150-178

Linkage assignment is based on [a]Flandez-Galvez et al. (2003); [b]Cho et al. (2004); [c]Winter et al. (2000). PIC - polymorphic information content

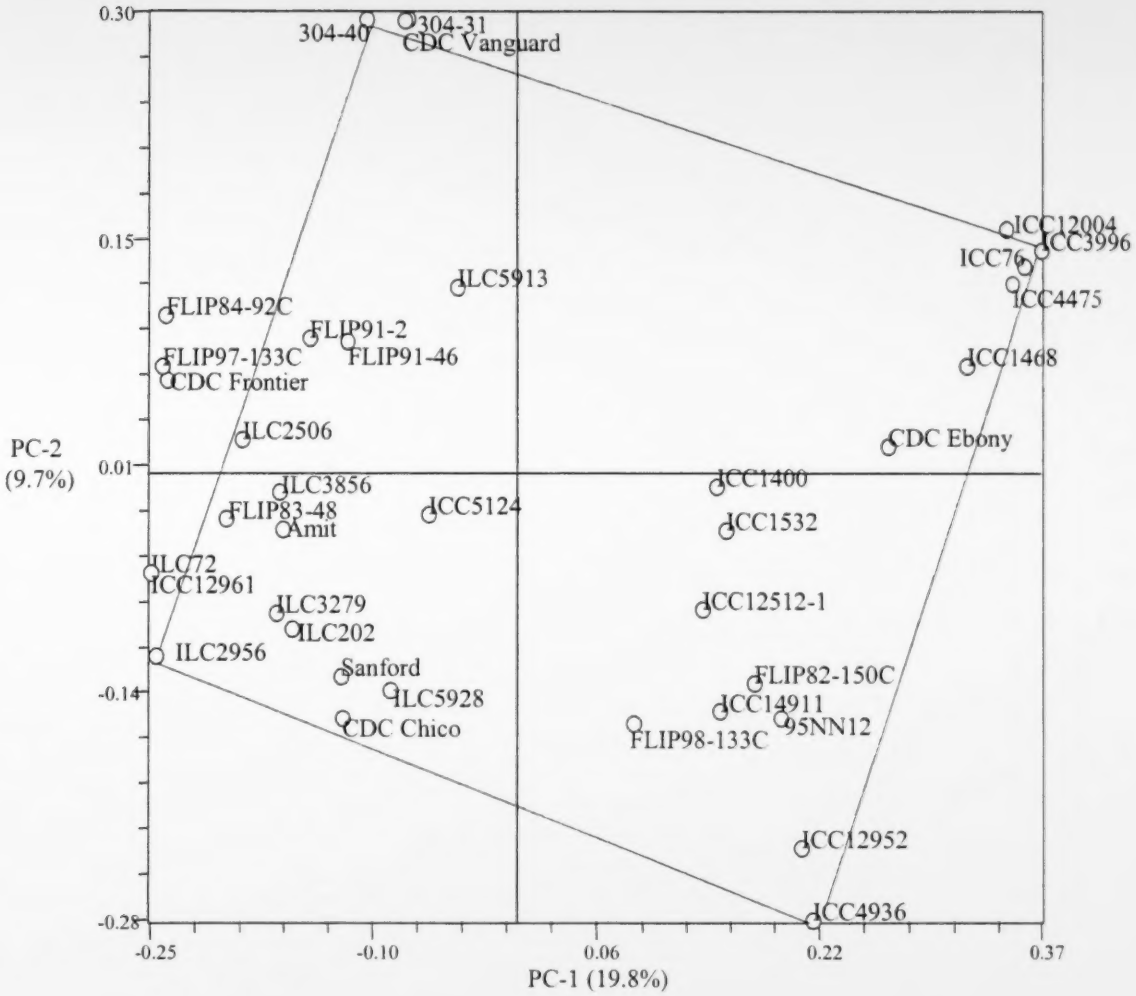


Fig. 6. Plot of the first and second principal coordinates based on the genetic similarity estimates of 37 chickpea genotypes differing in reaction to ascochyta blight. The most genetically distinct genotypes from each quadrant are indicated and connected by dotted lines.

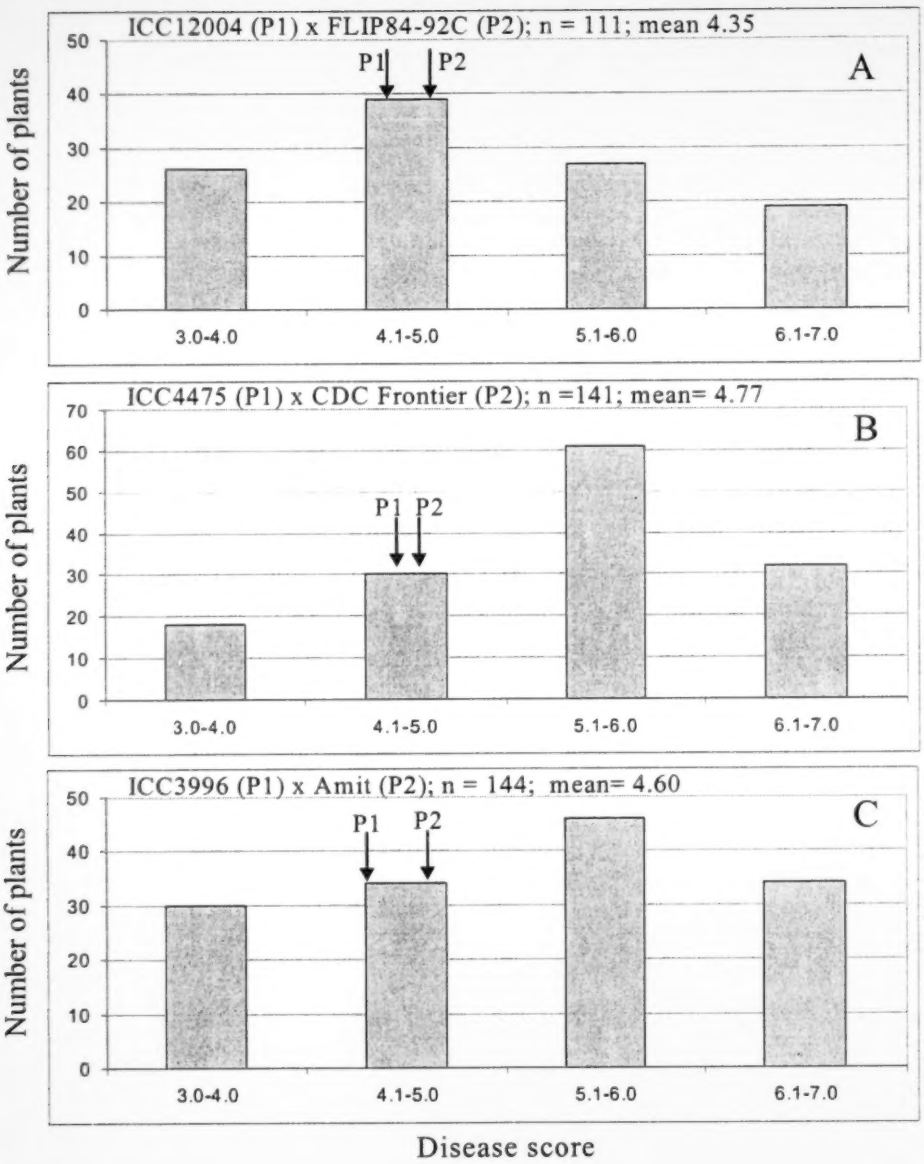


Fig. 7. Frequency distributions of three F_2 populations of chickpea derived from crosses of moderately resistant parents (A= ICC12004 x FLIP84-92C; B = ICC4475 x CDC Frontier; C = ICC3996 x Amit). The number of F_2 plants (n) for each population and mean disease score for each population are presented. Arrows show disease score for each parental line in each population. The disease was rated using a 0 to 9 scale, where 0 = no symptoms and 9 = plant dead

THEME IV. Identification and transfer of ascochyta resistance genes from related wild species to the cultigen

A few accessions of *C. bijugum* (ILWC260) and *C. judaicum* (ILWC164 and ILWC165) consistently showed higher levels of resistance to AB than the cultivated varieties. These accessions were selected as targets for interspecific hybridization with the cultivated species in collaboration with Dr. Monika Lulsdorf's laboratory. Over 6,000 crosses were attempted between the cultivated chickpea and accessions of *C. bijugum* and *C. judaicum*. However, only a few viable hybrids were obtained and subsequent hybrid growth was poor. It is clear that post-fertilization barriers such as embryo abortion, lack of endosperm initiation and/or endosperm breakdown limit the success of interspecific hybridization. Improvements to the tissue culture protocols are necessary in order to be able to use *C. bijugum* and *C. judaicum* as sources for ascochyta blight resistance.

The following putative hybrids were obtained from crosses between cultivated chickpea varieties and *C. bijugum* or *C. judaicum*: Number in brackets indicates the cross number.
(1) ICCV10 x *C. bijugum* ILWC 260 (D786)
(2) Sonali x *C. bijugum* ILWC 260 (H 565)
(3) Macarena x *C. bijugum* LIWC 260 (L350)
(4) ICCV 10 x *C. bijugum* LIWC 260 (M25)
(5) Sonali x *C. judaicum* ILWC 165 (M567)
(6) Sonali x *C. judaicum* ILWC 165 (M568)
(7) Macarena x *C. judaicum* ICC 17196 (I327)
(8) Macarena x *C. judaicum* ICC 17196 (K830)

Hybrid shoots were micro-propagated in order to conduct rooting experiments. To date, very few hybrid shoots could be rooted due to their lack of vigor, regardless if rooting media or grafting protocols were used.

The following bridge crosses were also made: *C. arietinum* x *C. echinospermum* and *C. arietinum* x *C. pinnatifidum*. Even with these bridge crosses, a relatively low frequency of F1 recovery was obtained. F_1 plants from these crosses were then crossed with *C. bijugum* or *C. judaicum*. In addition, crosses were also made between *C. pinnatifidum* x *C. judacium* and subsequently crossed with *C. arietinum*. However, no viable embryos/plants could be obtained from any of the bridge-crosses.

THEME V. Integration of ascochyta blight resistance into the CDC chickpea breeding program

The genotypes developed/identified in this project were crossed with adapted breeding lines from the CDC breeding program as a means to integrate the results of the current research into the breeding stream. Both simple (bi-parental) and complex (using 3 or more parents) crosses were performed. The first field evaluation of the progeny from these crosses was done in 2007 at the breeding nursery at Kyle, SK. Selection was done based on reaction to ascochyta blight, early maturity and seed quality. A total 156 $F_{4:5}$

families were selected and will enter the first yield trial at two or more locations in the 2008 growing season. Additional selected F_3 families across 85 populations will also be tested at Kyle as micro plots (Table 6).

Table 5. Selected F_5 families derived from pyramiding crosses involving 5 to 6 parents with diverse SSR alleles at the QTL for resistance to ascochyta or derived from bi-parental or three-way crosses for improvement of resistance to ascochyta blight for the first year yield trial across three or more locations

2008 test	Name	Pedigree	Number of selected lines	Type	2008 Location
CPYT 02	CA03-71	ICC12004/ILC3279//Frontier	7	K	Kyle, Pasqua, SPG
CPYT 04	CA05-75	(ICC12004/ILC3279)///(FLIP 97-133C/Amit)//Frontier	61	K	Kyle, Pasqua, SPG, Brooks
CPYT 05	CA03-45	Frontier/Amit	18	K	Kyle, Pasqua, SPG
CPYT 05	CA03-47	FLIP97-133C/Amit//Frontier	11	K	Kyle, Pasqua, SPG
CPYT 07	CA03-48	FLIP97-133C/ICC12512-1//Amit	4	K	Kyle, Pasqua, SPG
CPYT 09	CA03-59	FLIP97-133C/Frontier	27	K	Kyle, Pasqua, SPG, Brooks
CPYT 10	CA05-73	(FLIP 97-133C/ICC 12512-1)//Amit///(ICC12004/ILC3279)//Frontier	26	K	Kyle, Pasqua, SPG, Brooks
CPYT 12	CA03-70	ICC12512/ICC3996	2	D	Kyle, Pasqua, SPG, Brooks
		Total	156		

Table 6. Eighty-five populations derived from crosses of selected lines developed/ identified in this project (in parenthesis) with adapted materials from the CDC chickpea breeding program.

No	Cross	Pedigree	Seed type
1	AB06-1	(FLIP97-133C//ICCV96029/FLIP97-133C[BC1])///(ICC12512-1/Amit)	K
2	AB06-3	(FLIP97-133C//ICCV96029/FLIP97-133C[BC1])///FLIP82-150C	K
3	AB06-5	(ICC12512-1/Amit)//(ICC3996/FLIP97-133C)	D
4	AB06-6	(ICC12512-1/Amit)//CDC Xena	K
5	AB06-12	172-7//(FLIP94-48C/ICCV 96029)	K
6	AB06-13	172-7//(S95420/ICCV 96029)	K
7	AB06-18	(ICC3996/FLIP97-133C)//(ICC12512-1/Amit)	K
8	AB06-20	(ICC3996/FLIP97-133C)//95NN12	K
9	AB06-27	(FLIP94-48C/ICCV96029)//(Frontier/Amit)	K
10	AB06-37	(FLIP94-48C/ICCV96029)//95NN28	K
11	AB06-38	(FLIP94-48C/ICCV96029)//97-Indian2-125	K
12	AB06-39	(FLIP94-48C/ICCV96029)//FLIP82-150C	K
13	AB06-45	(S95420/ICCV96029)//95NN12	K
14	AB06-56	(ICC3996/FLIP97-133C)//97-Indian2-125	K
15	AB06-62	FLIP82-150C//(ICC3996/FLIP97-133C)	D

16	AB06-64	FLIP98-136C//(Frontier/Amit)	K
17	AB06-68	(Ca05-75)/491-10	K
18	AB06-70	438-29/(FLIP98-130C)	K
19	AB06-78	(ICC12512-1/Frontier)//491-16	K
20	AB06-81	(ICC12512-1/Frontier)//(FLIP98-142C)	K
21	AB06-86	(FLIP97-133C/Amit)//491-11	K
22	AB06-91	(S95420/ICCV96029)//(ICC12512-1/Frontier)	K
23	AB06-92	(S95420/ICCV96029)//439-22	K
24	AB06-93	(S95420/ICCV96029)//491-10	K
25	AB06-94	(S95420/ICCV96029)//FLIP00-44C	K
26	AB06-95	(S95420/ICCV96029)//FLIP98-135C	K
27	AB06-96	(ICC3996/FLIP97-133C)//FLIP97-133C	K
28	AB06-100	FLIP97-133C / (FLIP97-95C)	K
29	AB06-102	(FLIP98-121C)/491-11	K
30	AB06-103	(FLIP98-131C)/382-1	K
31	AB06-104	(FLIP98-131C)/(FLIP97-95C)	K
32	AB06-106	(FLIP98-131C)/491-11	K
33	AB06-107	(FLIP98-142C)//(ICC12512-1/Frontier)	K
34	AB06-108	(FLIP98-142C)/491-11	K
35	AB06-110	(FLIP98-142C)/441-34	K
36	AB06-112	(FLIP98-142C)/FLIP97-101C	K
37	AB06-115	491-10/(FLIP98-142C)	K
38	AB06-117	(FLIP95-55C)//(ICC12512-1/Amit)	K
39	AB06-118	(FLIP95-55C)/491-16	K
40	AB06-119	(FLIP95-55C)/491-11	K
41	AB06-120	493-29 / (FLIP97-137C)	K
42	AB06-122	493-29 / (FLIP98-121C)	K
43	AB06-123	493-29/(FLIP97-657C)	K
44	AB06-129	493-7//(ICC3996/FLIP97-133C)	K
45	AB06-132	382-1//(ICC3996/FLIP97-133C)	K
46	AB06-136	491-16//(ICC12512-1/Amit)	K
47	AB06-138	491-16//(FLIP97-133C/Amit)	K
48	AB06-140	491-16 / ((FLIP98-121C))	K
49	AB06-141	491-16 / (FLIP97-95C)	K
50	AB06-142	491-16 / (PCh5)	K
51	AB06-144	491-16/((FLIP95-55C))	K
52	AB06-146	491-11//(S95420/ICCV96029)	K
53	AB06-147	491-11/((FLIP98-131C))	K
54	AB06-148	491-11/(FLIP97-657C)	K
55	AB06-150	491-11/(FLIP98-130C)	K
56	AB06-151	491-11/(FLIP98-142C)	K
57	AB06-153	491-11/(FLIP95-55C)	K
58	AB06-154	491-11/(FLIP97-95C)	K
59	AB06-156	491-11/FLIP97-101C	K
60	AB06-162	FLIP97-101C//(FLIP97-133C/Frontier)	K
61	AB06-166	FLIP97-101C//(ICC12512-1/Amit)	K
62	AB06-167	FLIP97-101C//(ICC12512-1/Frontier)	K
63	AB06-169	FLIP97-101C//(FLIP97-133C/Amit)	K
64	AB06-175	Y9463-028//(ICC12512-1/Amit)	D
65	AB06-176	Y6172-011//(ICC12512-1/FLIP97-133C)	D

66	AB06-178	452-2//(ICC12512-1/Amit)	D
67	AB06-181	425-14//(ICC12512-1/FLIP97-133C)	D
68	AB06-182	425-14//(ICC12512-1/Frontier)-17	D
69	AB06-183	425-14//(ICC12512-1/Frontier)-59	D
70	AB06-184	425-14//(ICC12512-1/Amit)-248	D
71	AB06-185	425-14//(ICC12512-1/Amit)-60	D
72	AB06-189	425-14//(ICC12512-1/Amit)-52	D
73	AB06-190	512-51//(ICC12512-1/Amit)-110	D
74	AB06-192	512-51//(ICC12512-1/Amit)-77	D
75	AB06-195	512-51//(ICC12512-1/Amit)-146	D
76	AB06-196	512-51//(ICC12512-1/Amit)-52	D
77	AB06-197	(ICC12512-1/FLIP97-133C)// Y6172-011	D
78	AB06-198	(ICC12512-1/FLIP97-133C)//452-2	D
79	AB06-201	(ICC12512-1/Frontier)//(S95420/ICCV96029)	D
80	AB06-202	(ICC12512-1/Frontier)//Y9463-028	D
81	AB06-205	(ICC12512-1/Amit)-110//425-14	D
82	AB06-206	(ICC12512-1/Amit)-248//Y6172-011	D
83	AB06-207	(ICC12512-1/Amit)-52//Y9463-028	D
84	AB06-208	(ICC12512-1/Amit)-52//Y6172-011	D
85	AB06-209	(ICC12512-1/Amit)-52//452-2	D

This research indicated that further improvement in resistance to ascochyta blight is feasible in chickpea. Combination strategies of exploitation of the variation within the cultivated species and introgression of resistance genes from related wild species could be used to make substantial genetic gain. Efforts to transfer the resistance from the wild species such as *C. bijugum* and *C. judaicum* must continue. The realization of this approach, however, will be in the very long term. In the medium to long term, non-conventional or new means of improving the resistance should be explored. This may include inserting foreign gene(s) related to resistance to fungal pathogens, or modifying the current resistance genes once better understanding of their molecular, biochemical and metabolic functions is known.

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Papers/Presentations:

Two manuscripts indicated below were published in refereed journals and two others are in preparation.

1. Tar'an, B., T. Warkentin, A. Tullu and A. Vandenberg. 2007. Genetic relationships among chickpea (*Cicer arietinum* L.) genotypes based on the SSRs at the quantitative trait loci for resistance to ascochyta blight. European J. Plant Pathology 119: 39-51.
2. Tar'an, B., T. Warkentin, A. Tullu and A. Vandenberg. 2007. Genetic mapping of ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using a simple sequence repeat linkage map. Genome 50: 26-34.

Poster and oral presentations were given at several international meetings. In each meeting/presentation financial support from Saskatchewan Agriculture and Food and the Saskatchewan Pulse Growers was gratefully acknowledged.

(i) Anbesa, Y., B. Tar'an and T. Warkentin. 2007. Identification of QTLs conferring resistance to ascocyta blight in chickpea. North American Pulse Improvement Association meeting, November 1-2, 2007, Madison, WI.

(ii) Tar'an, B. T. Warkentin, A. Tullu and A. Vandenberg. SSR-based Haplotype Diversity at the QTL for Ascochyta Blight Resistance in Chickpea. 1st International Workshop on Ascochyta of Grain Legumes, July 2 – 6, 2006, Le Tronchet, Rennes, France.

(iii) Tar'an B, Warkentin T, Lulsdorf M, Banniza S, Tullu A, Bett K, and Vandenberg A. Breeding for higher levels of resistance to ascochyta blight in chickpea. 4th International Food Legume Research Conference (IFLRC), 18-22 October 2005, New Delhi, India.

(iv) Tar'an B, Warkentin T, Lulsdorf M, Banniza S, Tullu A, Bett K, and Vandenberg A. Breeding for higher levels of resistance to ascochyta blight in chickpea. North

American Pulse Improvement Association meeting October 28 - November 3, 2005, Delaware, USA.

Information of benefit to producers, processors, or governments:

The results of classical and molecular genetics experiments demonstrated that a few genes control resistance to AB in chickpea. CDC Frontier, which has an equal level of resistance to that of the best varieties released in the world, carries three QTL for resistance. It appears possible to gradually increase the level of resistance beyond that of CDC Frontier by accumulating resistance alleles from different sources that are genetically distant from CDC Frontier. Further, quantitative genetic analyses estimated that the gain in selection for AB resistance varied around 10% from crosses involving moderately resistant parents. Using various sources of resistance available in the cultivated species of chickpea, it should be possible to improve the resistance levels to around 3.0 to 3.5 on a 0 –9 scale. It appears unlikely/very difficult to improve the resistance below the 3.0 level by this approach. Efforts to transfer the resistance from the wild species such as *C. bijugum* and *C. judaicum,* which have higher resistance than in cultivated species, must continue. The realization of the latter approach, however, will be in the very long term.

(d) Personnel:

Dr. Yadeta Anbessa Kabeta was hired in September 2006 to replace Dr. Tar'an on the day-by-day activities of this project, while Dr. Tar'an continued to supervise and monitor the research progress. Mrs. Parvaneh Hashemi continued to assist in disease screening and plant maintenance in the greenhouse and phytotron as well as to help with some aspects of marker analyses.

(e) Equipment purchased:

None

(f) Project Developed Materials:

The list of materials that were developed from this project is as follows:

- 10 experimental populations (from R x S and R x R crosses; Table 1) which will be invaluable resources for future research involving ascochyta blight resistance in chickpea and other traits segregating in the populations.
- SSR based linkage map. Since most of the markers are common, this map can be used as a reference to align several existing maps developed using different populations.
- 156 F4 and F5 lines derived from pyramiding crosses involving multiple parents with diverse SSR alleles at the QTL for resistance to ascochyta blight. These lines

will enter the first year yield trial at multi locations in 2008 (Table 5). Any superior lines from these materials will be selected and used as parents for the future breeding and/or released as new variety.

- 85 F3 populations developed from various crosses involving the materials developed/ identified in this project with the adapted lines from the CDC breeding program (Table 6).

(g) Project Photos:

Numerous photos from disease screening in the field and greenhouse have been taken and could be provided if needed. Also numerous gel pictures of the SSR diversity have been taken. Some photos are included in the presentations and manuscripts arising from this research.

(h) Acknowledgement:

The Saskatchewan Agricultural Development Fund and the Saskatchewan Pulse Growers were acknowledged in all presentations where this research project was mentioned.

(i)Expense Statement:

Will be provided.

(j) ICAR Data Entry

Will be entered.